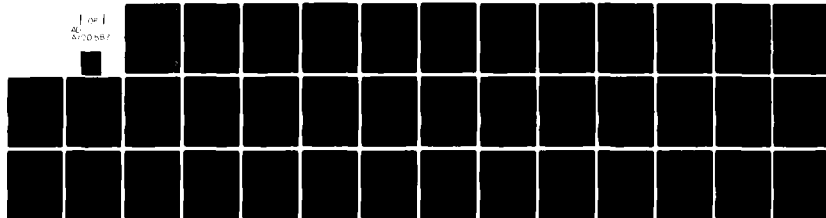


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NATIONAL BUREAU OF STANDARDS WASHINGTON DC CHEMICAL --ETC F/G 13/2
ULTRATRACE SPECIATION AND BIOGENESIS OF METHYLtin TRANSPORT SPE--ETC(U)
1981 F E BRINCKMAN, J A JACKSON, W R BLAIR N00014-81-F-0013
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ULTRATRACE SPECIATION AND BIOGENESIS OF METHYLTIN TRANSPORT SPECIES
IN ESTUARINE WATERS

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ABSTRACT

Environmental tin, widely dispersed at low concentrations in waters, sediments, and biota, is now perceived to be a bioactive element susceptible to methylation and even hydridization by marine bacteria. Nonetheless, the redox cycle of tin in natural waters is poorly understood and recent advances in tin-specific molecular characterization fail to speciate Sn(II) and Sn(IV) reliably. On the other hand, such rapid developments in speciation methodology now permit growing numbers of studies of organotin distributions in aquatic systems, raising the question of the "natural" biogeochemical flux of methylstannanes in relation to increased anthropogenic organotin influx from industry and shipping. New methods for direct speciation of aquated or involatile organotins by liquid chromatography are compared with advances in purge-and-trap sampling of volatile or hydrophobic organotins speciated by gas chromatography. The work in our laboratory indicates that effective models for estuarine formation and transport may ultimately be developed, but that basic roadblocks to progress stem from inadequate descriptive aqueous organometallic chemistry and knowledge of critical kinetic parameters for the lifetimes of key organotin species in sea water.

Key words: Atomic absorption detector; biomethylation; Chesapeake Bay; estuarine organotin transport; flame photometric detector; gas chromatography; kinetics; liquid chromatography; methyltin hydrides; purge-and-trap sampling; redox; tetramethyltin.

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ULTRATRACE SPECIATION AND BIOGENESIS OF METHYLtin TRANSPORT SPECIES IN ESTUARINE WATERS

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INTRODUCTION

Tin is very widely dispersed in the aquatic environment, appearing as trace amounts in sediments, particulates, and biota (mg kg^{-1}), and at much lower levels in waters (ng L^{-1}) [1]. The concentration of tin decreases notably from estuarine and near shore sites to oceanic environments [2], in a manner seen for other metals or metalloids subject to significant anthropogenic inputs and rapid geochemical turnovers and transport [3].

The chemistry of tin in natural waters is generally presumed to be the stannic or tin(IV) species, but its redox chemistry in sea water remains largely unclear, as does its biogeochemistry. This last aspect has recently assumed great importance in understanding the mechanisms by which aquatic tin is mobilized (by atmospheric or aquatic transport) and in questioning both bioavailability and bioaccumulation of tin [4,5]. Several reports on the biomethylation of tin in both inorganic and organometallic forms have now appeared [2,6-9], and ubiquitous distributions of methyltin(IV) species at ultratrace concentrations (ng L^{-1}) in natural waters have been observed [10-12]. This evidence suggests that

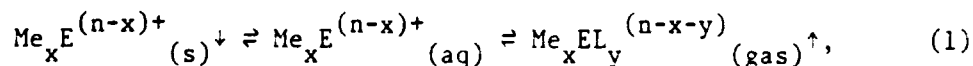
*National Research Council-National Bureau of Standards Postdoctoral Research Associate, 1979-1981.

such molecules may take part in a global tin cycle. Unfortunately, the matter is obscured by the sharp increase over the past twenty-five years of anthropogenic introduction of both methyl- and other organotins into the environment by diverse commercial materials [13].

Interactions of man-made organotin compounds intrude into the "natural" biogeochemical cycle of tin [14] in special ways. Use of organotins mainly occurs as plastics stabilizers and biocides; these, in turn, find principal applications in ways that involve environmental exposure, especially in fresh and estuarine discharges. We have undertaken studies to speciate tin and to assess its dynamics in the marine environment, but in view of the foregoing situation we are examining the tin concentration gradient from a well-known estuarine source, the Chesapeake Bay, to oceanic sites. In this context, we will discuss progress in current research to solve fundamental problems impeding progress toward resolving the role of tin in the sea. The objectives of this research have been to: (1) establish a firm data base for the aqueous chemistry of organotin(IV), tin(IV), and tin(II) species, including dynamics of their interactions with other trace aquated metal ions; (2) develop and apply reliable ultratrace molecular speciation techniques to the measurement of aquatic tin in its various forms, including key, volatile transport species such as tetramethyltin; and (3) assess the role of marine microbiota in generating or retarding formation of tin transport species, by biosynthesis, biodegradation, and uptake.

BIOAVAILABILITY OF TIN IN RELATION TO OTHER BIOACTIVE ELEMENTS

Several estimates of global transport cycles suggest [15,16] that atmophilic properties of certain elements may, in part, depend upon biological mediation. For a given element, such volatilization processes basically must rely upon release from terrestrial or aquatic sediment reservoirs of hydrophobic gases such as elemental mercury, Hg^0 , or coordinatively saturated methylelements, Me_nE^0 , where n is the principal oxidation state [5]. In essence, the underlying chemical processes governing retentivity or transport to/from sediments or particulates to/from water columns to/from atmosphere are,



where L^- = some anionic ligand imparting volatility, such as H^- , Me^- , Cl^- , or OH^- . Below, we shall review the features of such derivatization chemistry as a means to produce water-air partition coefficients favorable for development of analytical methods employing chromatographic separations coupled with element-selective detection [17]. A perspective on mobilization of tin is gained by comparing its non-specific (total) availability in common environmental reservoirs in relation with other bioactive elements known to undergo transformations of the kind implied by equation (1).

Potential Availability of Tin in Environmental Media

Table 1 summarizes data from various sources which suggest that considerable amplification of many such active elements can occur. Sewage sludges from 16 American cities show [18] substantial industrial and microbial accretion of many heavy elements from common sources (cf. cow manure) into forms that may offer enhanced availability to subsequent biota during translocation via stream loadings to estuaries and then to the sea. It is tempting to associate the very high concentrations of biogenic cobalamins (vitamin B_{12} derivatives) observed during sewage sludge treatment (1.9 to $27.1 \mu g g^{-1}$) [24] with potential involvement of a powerful methylator, methylcobalamin or CH_3-B_{12} [14], in metal release.

Though not yet identified in sewage sludges, presence of CH_3-B_{12} , especially in anaerobic sediments, is reasonable. Consequently, the effectiveness with which this exocellular metabolite methylates a broad range of metal ions in solution [14,25] suggests an important new line of investigation for metal release from sewage outfalls. Moreover, Thayer reports [26] that CH_3-B_{12} is also capable of dissolving many refractory metal oxides, including highly insoluble SnO_2 , to possibly form methylmetal species in aqueous solution.

Similar considerations, doubtless for small chemical concentration gradients, probably are valid for some of the other tin sources listed in table 1. The very high tin concentrations (also associated with heavy loadings of organic matter) in the Baltimore Harbor sites focused the direction of our present work. Moderately high concentrations of total tin in sub-micron materials from the Chesapeake Bay channel represent favorable surfaces for attachment and transformation of metals by marine bacteria [27]. In table 1, no

Table 1. Compositional Data^a for Tin and Some Other Bioactive Elements in Various Environmental Sources

Source	As	Hg	Pb	Se	Sn
Cow manure ^b	4.0	0.2	16.2	2.4	3.8
Sewage sludge ^b	3.0-10.0	3.4-18.0	136.0-7627.0	1.7-8.7	111.0-492.0
Urban dust ^c	115.0	--	0.655 ^d	24.0 ^e	--
U.S. water supplies ^f	0-100.0	0.01-30.0	1.0-400.0	0-10.0	0.3-30.0
Chesapeake Bay					
Baltimore Harbor sed.	1.1 ^g	<0.01-0.31 ^h	<1-1.38 ^{d,h}	--	1.8 ^g -240.0 ⁱ
Baltimore Harbor waters	0.29 ^g	<0.071 ^g	1.9 ^g	--	0.19 ^g -<2.0
Channel sed. ^j	0.92-10.0	<0.065-0.37	0.54-75.0	--	0.19 ^g -1.7
Channel waters	<0.079-0.37	<0.038-0.16	0.10-1.9	--	<0.13-0.47
Organic colloids ^j	3.0-4.0	--	--	0.65-2.0	54.0-57.0

^a Individual, mean, or range values in $\mu\text{g g}^{-1}$, dry weight (solids), or $\mu\text{g l}^{-1}$ (waters).

^b Ref. 18

^c Given as Σ Pb

^d Ref. 19

^e Ref. 20, outer harbor station

^f Ref. 21

^g Ref. 22, S-N Bay axis, 22 stations

^h Ref. 23

^c NBS Standard Reference Material 1648

^e Not certified

^g Ref. 20, outer harbor station

ⁱ Ref. 2

^j Unfiltered

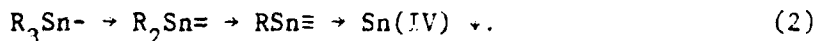
mention of another very important microenvironment for amplification of metals is given. Heavy elements are well known to greatly concentrate in oily surface microlayers [28], but data for total or speciated tin in the Chesapeake Bay are not yet available.

Anthropogenic Introduction of Organotins into Environmental Media

In the foregoing we have discussed the typical total tin content of environmental compartments involved in transmission of this element (and related bioactive elements) from land to sea. The rich chemistry of tin has made this element one of great importance to man's industries for millenia, and in recent decades its remarkable range of properties as organotin compounds has generated totally new markets and distribution patterns in the environment [13].

The contribution of all anthropogenic tin compounds represents the bulk of the tin discharge system to oceans [16]. Over four percent of this contribution involves organotins of widely varying toxic properties, particularly toward aquatic biota. Table 2 indicates the spectrum of organotin formulations involved. The R_3Sn- class is the most toxic generally, but the $R_2Sn=$ species represent still lipophilic moieties that can probably undergo transformations in vivo to create toxic responses within biota, as we shall see later.

It is well known that, in addition to the degree of substitution on tin(IV), the precise molecular structure of the substituent group R covalently bound to the organotin influences its lethal action in different categories of organisms [29]. Nonetheless, most investigators now agree that biodegradation of even toxic R_3Sn -containing materials can occur in soils and sediments, presumably caused by bacteria that successively cleave tin-carbon bonds to ultimately produce inorganic tin:



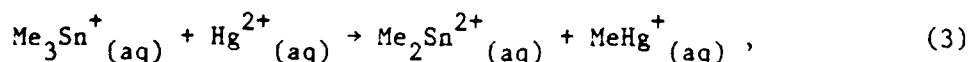
In practical fact, these steps are assumed, there having been no clearcut demonstration of the entire sequence in the environment. Moreover, the new evidence for microbial methylation of inorganic tin(IV), tin(II), and common intermediate organotins suggests--just as was shown earlier for the sedimentary cycle for methylmercury [26,30,31]--that a counter mechanism for sustaining methyltin(IV)

Table 2. Production of Organotins and Their Uses^{a,b}

Uses	Chemical Form ^c	Relative %
PVC Plastics (heat stabilization)	R_2SnL_2	66.4
Catalysts (polyurethanes, silicone elastomers)	R_2SnL_2	20.7
Biocides (pesticides, antifoulants)	R_3SnL and $\begin{array}{c} -[L]- \\ \\ SnR_3 \end{array}$	8.3
Miscellaneous (research, fiber stabilization, export)	R_3SnL	3.3
Poultry (anthelmintics, coccidiostats)	R_2SnL_2	1.2

^aRef. 13^bRepresents 4.2% of 1976 total world production, 2.26×10^{11} g^cR = butyl, cyclo-hexyl, phenyl, octyl, methyl, and propyl; L = F, Cl, laurate, maleate, and thioglycolate.

species in environmental media is available. The reported [10-12] widespread incidence of highly diluted solvated methyltin(IV) ions, $Me_nSn^{(4-n)+}$ (aq), in United States coastal waters may therefore simply reflect a steady state concentration. The idea that this situation is reached only through biological mediation is not valid, however. Brinckman has pointed out [5] that among the methyltin species identified in marine waters, Me_3Sn^+ (aq) is itself a potent methylator of many aquatic metal ions at bimolecular rates which might significantly shift the apparent "steady-state" concentration. For example, with aqueous mercuric ion, the reaction



proceeds to completion at a rate first-order in each metal and dependent on the $[Cl^-]$ and ionic strength of the solution [32].

Based on available concentrations of free $Hg^{2+}_{(aq)}$ and $Me_3Sn^{+}_{(aq)}$, along with typical pCl, pH, and ionic strengths for coastal waters, it was estimated [5] that the half-life for leakage of Me_3Sn^{+} from that compartment was about 6×10^5 yr. This residence is in surprising agreement with that estimated for oceanic total tin, 1×10^5 yr [33], but must be fortuitous since many other abiotic transmethylation pathways for forming and decomposing methyltins are known. We shall consider several of these possibilities in the following section, both in the context of biomethylation and abiotic transmethylation.

CHEMICAL AND BIOLOGICAL INTERACTIONS OF METHYLTINS WITH ELEMENTS

Within a microenvironment, concurrent events involving both chemical and biological reduction and methylation of metals may transpire individually or in synergic fashion. Figure 1 illustrates a combination of in vitro and environmental experience for three heavy elements of great technological importance that undergo microbial methylation in marine sediments [26]. No evidence is available for common enzymes among the bacterial populations that methylate all three elements, but it is reported that pure strains of genus Pseudomonas, for example, can both reduce and methylate As(V) to As(III) and form Me_nAsH_{3-n} gases [34], reduce Hg(II) to Hg^0 [35], and both methylate and reduce Sn(IV) to form Me_nSnH_{4-n} gases [5,12]. Together, these metabolites or their precursor metal species may also interact in situ. The case for a mercury-tin "crossover" model has been described [6,36] where combined stresses of Sn(IV) and Hg(II) (a much more realistic representation of polluted sediments!) were metabolized by a metal-tolerant strain of Pseudomonas isolated from the Chesapeake Bay [35]. The metabolic reduction of mercury to Hg^0 gas and the biomethylation of tin(IV), seen to occur for the respective metals as individual stresses, were seen to occur with the combined stress. A new species was formed only during metabolism of the dual metal stress. This species was found to be $MeHg^{+}$, presumably formed by the reaction given in equation (3),

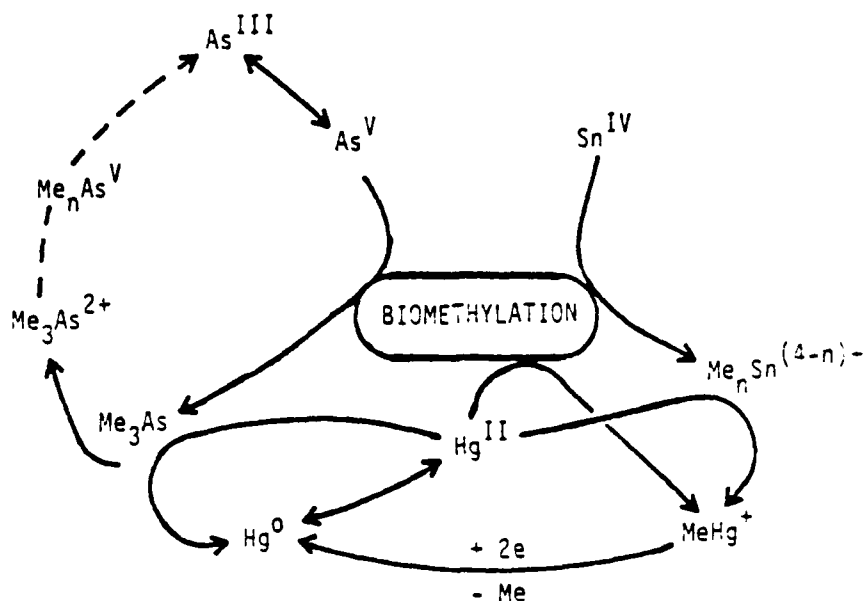
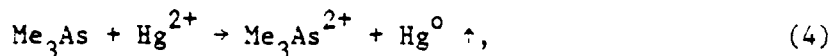


Fig. 1. Associated abiotic chemistries of three elements known to undergo biomethylation in marine sediments are compared.

where it was concluded that both concurrent biomethylation of tin(IV) and its mediation (abiotically) in MeHg^+ production were kinetically competitive [6].

Other dual metal crossovers are possible in principle. We examined the possibility for $\text{Me}_3\text{As}^{2+}(\text{aq})$, an isoelectronic analog of $\text{Me}_3\text{Sn}^+(\text{aq})$, for its ability to methylate metal electrophiles, in particular Hg^{2+} [37]. As depicted in figure 1, only a redox reaction in the reverse sense occurred reducing arsenic volatility,



which was highly dependent upon pH and ligands bound to mercury [38]. Such chemistry is important to considerations of microbial production of Me_3As [34] in sediments where high concentrations of both As and Hg are commonly found (cf. table 1). Similar experiments with arsenic and tin species in saline waters at these pH and pCl conditions gave no conclusive reactions.

The abiotic organometallic reactions noted above which readily occur in aqueous solutions represent only a few of many similar redox and transmethylation processes recently described for other metals in this expanding field [5,26]. To aid in understanding our recent results in speciation and dynamics of methyltins in estuarine waters, brief mention of a group of abiotic reactions described for both environmental and laboratory conditions is desirable. In table 3, we summarize some representative reactions which either favor formation of new methylelement species potentially capable of biotic uptake, or elimination of "active" methyl groups from aquatic environments and potential biotic activity. It is clear from the table that ubiquitous coastal production of MeX [15,44] or photo-methylation via metabolic acetates represent possible sources of environmental methylelements, and thus merit further study. All of the aqueous reactions cited in table 3 probably occur at bimolecular (or pseudo first-order) rates of the order, $k \sim 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$ or less [5], but like the related transmethylation reaction illustrated in equation (3) probably can kinetically compete with microbial methylation and demethylation reactions [26].

SPECIATION OF ORGANOTINS IN SEA WATER*

Requirements for Nondestructive Methods

Beyond the usual difficulties encountered with determinations of metals at ultratrace (ng L^{-1}) concentrations in biological fluids and aquatic media [45], creation of a realistic framework of data for establishing the role of tin in the sea requires molecular speciation as well. We have examined the interdependence of biological and chemical processes for transformation and mobilization of tin and other metals and have noted that rate-determining events depend upon very specific features of molecular architecture and oxidation state of the associated metal ion. Thus, the number and kinds of organic ligands bonded to tin(IV) are not only diagnostic of toxicity, but they also provide a molecularly distinctive analyte for elucidation of trace chemistry, if suitable nondestructive chemical or physical means for isolation, concentration, and unambiguous detection can be applied.

* Complete descriptions of equipment and methods employed at NBS are given in references 12, 51, or 49, 50 for gas and liquid speciation, respectively.

Table 3. Some Non-biological Reactions^a Providing Formation or Removal of "Active" Methyl Groups from Aquated Metal Ions

Formation Reactions	Reference
$\text{MeX}^b + \text{Sn}^{2+} \rightarrow \text{Me}_n\text{Sn}^{(4-n)+} + n\text{X}^-$ $(\text{X} = \text{F}, \text{Cl}, \text{MeCO}_2, \text{SO}_4) \quad (n = 1, 2)$	7
$\text{MeI} + \text{Pb}^{2+} \rightarrow [\text{MePbI}^{2+}] \rightarrow \text{Me}_4\text{Pb}$	39
$\text{MeX}^b + \text{Me}_3\text{E} \rightarrow \text{Me}_4\text{E}^+ + \text{X}^-$ $(\text{X} = \text{Br}, \text{I}; \text{E} = \text{As}, \text{Sb})$	40
$\text{MeCOO}^{-c} + \text{Hg}^{2+} + h\nu^d \rightarrow \text{MeHg}^+ + \text{CO}_2$	41, 42
Removal Reactions	
$\text{Me}_3\text{E}^+ + \text{M}'\text{Cl}_4^{2-} \rightarrow \text{M}'^0 + \text{MeCl} + \text{Me-Me} + \text{Me}_2\text{E}^{2+}$ $(\text{E} = \text{Sn}, \text{Pb}; \text{M}' = \text{Pd}, \text{Pt})$	42
$\text{Me}_3\text{E}^+ + \text{AuCl}_4^- \rightarrow \text{Au}^0 + \text{MeCl} + \text{Me}_2\text{E}^{2+} \quad (\text{E} = \text{Sn}, \text{Pb})$	42
$\text{Me}_4\text{Pb} + \text{Cu}^{2+} \rightarrow \text{CuCl} + \text{MeCl} + \text{Me}_3\text{Pb}^+$	43
$\text{MeHg}^+ + \text{PdCl}_4^{2-} \rightarrow \text{Hg}^{2+} + \text{MeCl} + \text{Pd}^0$	
$42\text{Me}_3\text{E}^+ + \text{TlCl}_n^{(3-n)-} \rightarrow \text{Tl}^+ + \text{MeCl} + \text{Me}_2\text{E}^{2+} \quad (\text{E} = \text{Sn}, \text{Pb})$	42
$\text{Me}_2\text{Hg} + h\nu^d \rightarrow \text{Hg}^0 + \text{Me-Me}$	41, 42

^aIn saline solutions

^bA common metabolite ($\text{X} = \text{Cl}, \text{Br}, \text{I}$) from bacteria and algae in coastal sea water, see Ref. 44

^cCommon bacterial metabolite

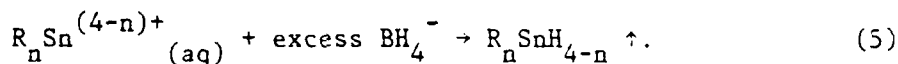
^dSunlight or ultraviolet irradiation.

Two basically different approaches are possible, and have been adopted in our present work. We recognize from equation (1) that organotin species of interest to us, $\text{R}_n\text{Sn}^{(4-n)+}$, may exist in equi-

librium between aqueous solution and complexes on solid substrates, such as inorganic particulates or biological detritus. Apart from those species already in homogeneous or "free" solution, a choice is possible for treatment with selective ligands, L, that can either cause desorption of solid-bound organotins, or, alternatively, form new organotin derivatives possessing necessary volatility (hydrophobicity) for recovery from solution as gases. In both of the methods to be described, we directly speciate aquated organotins--those in "free" solution--without pretreatment. For those organotin molecules of sufficient volatility, e.g., Me_4Sn , a gas chromatographic separation step is employed; for involatile solvated organotins, a direct liquid chromatographic separation process is utilized, and, when appropriate, hydride derivatization can be exploited with the gas chromatograph. Both methods give high precision retention times (RSD < 3 percent) with authentic organotins, a measurement prerequisite for reliable speciation.

Gas Phase Speciation of Aquatic Organotins

Braman [10] and Goldberg [11] with their students successfully speciated aquatic organotins ($R = \text{Me}$ and Bu), respectively, with flame emission (SnH) and atomic absorption detectors, following gas sparging of large (100 mL) water samples treated with a hydriding agent,



The resulting stannanes ($n = 0$ to 3) were cryogenically trapped, then distilled into the detector. Though extremely sensitive ($< 1 \text{ ng L}^{-1}$) the methods failed to isolate Me_4Sn or other volatile organotins not requiring hydride volatilization. We have modified commercial equipment to provide a system which avoids the above limitations, though it is not as sensitive.

Our system employs a "Tenax-GC" polymeric sorbent [46] in an automatic purge and trap (P/T) sampler coupled to a conventional glass column gas chromatograph equipped with a flame photometric detector (FPD). A schematic of the P/T-GC-FPD assembly with typical operating conditions is depicted in figure 2. Flame conditions in the FPD were tuned to permit maximum response to SnH emission in a H-rich plasma, as detected through narrow band-pass interference filters ($610 \pm 5 \text{ nm}$) [47]. Two modes of analysis were used: (1) volatile stannanes were trapped directly from sparged 10 to 50 mL

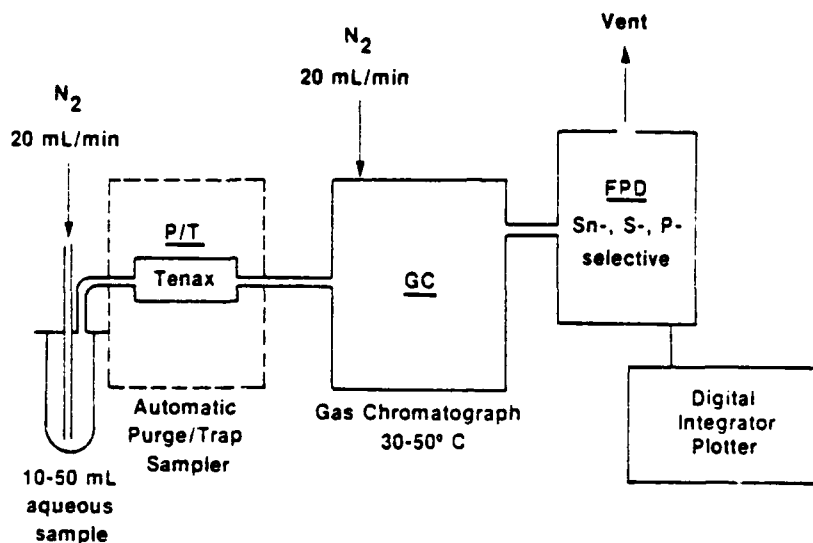


Fig. 2. The purge/trap GC-FPD system and operating conditions [12]

water samples with no pretreatment, and (2) volatilized tin species were trapped from the same or replicate water samples following rapid injection of aqueous excess NaBH_4 solution directly into the P/T sparging vessel immediately prior to beginning the P/T cycle [12].

Direct Speciation of Organotins in Solution

The liquid-solid solvation process illustrated in equation (1) also governs several desirable molecular separation processes offered by high-performance liquid chromatography, HPLC [48]. For either ion exchange resolution of aqueous cations, $\text{R}_n\text{Sn}^{(4-n)+}$, [49], or their separation as ion pairs, $[\text{R}_n\text{Sn}^{(4-n)+}\text{X}_{4-n}]^{\text{aq}}$, on reverse bonded-phase columns [50], the method is restricted to "free" tin analytes. Unlike the vigorous hydride derivatization used in the GC-FPD method,

common HPLC solvent combinations or their ionic addends usually will not provide sufficient coordination strength to labilize organotin ions strongly bound to solids in environmental samples. Moreover, the HPLC separations require that injected samples be free of particulates that may clog the column or pumping system.

On the other hand, HPLC, if coupled with a sensitive element-specific detection system such as atomic absorption spectrometry (AA), offers a valuable tool for organotin speciation in complex fluids (especially with high organic loadings) not readily amenable to gas phase derivatization methods. Figure 3 shows a schematic of

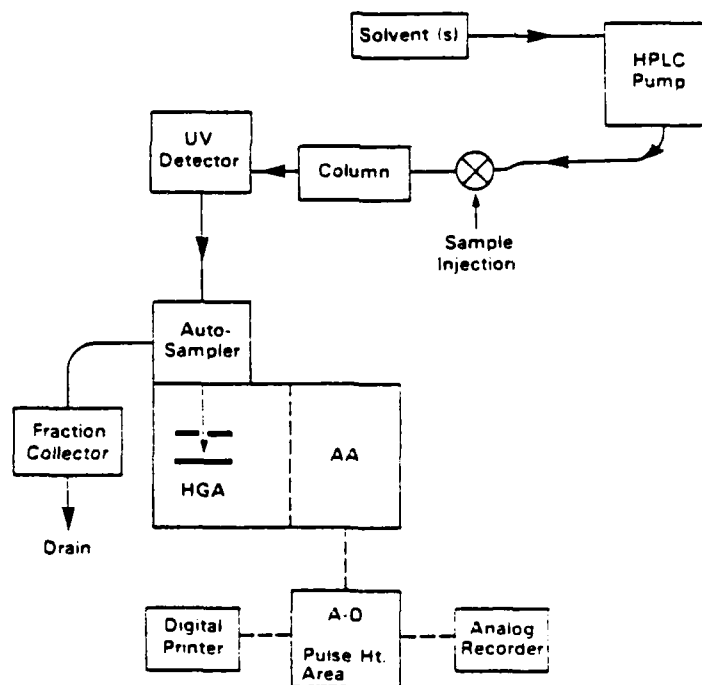


Fig. 3. The HPLC-GFAA system with automated peripherals [50]

our basic HPLC setup coupled to a graphite furnace AA (GFAA) in a manner giving automatic periodic (typically 45 s intervals) sampling of the resolved eluants for tin-specific determination [50]. Injected sample volumes may vary from 10 to 500 μL . Consequently, system sensitivity is broad and samples can be very representative. For example, direct examination of soil or sewage leachates, process waters, solutions exposed to organotin (antifoulant) controlled-release agents, or microbial growth media, only requires separation of particulates (or cells) prior to speciation. This is simply done by decantation, syringe filtration, or ultracentrifugation before direct injection of the solution into the HPLC-GFAA system. Thereby, needed information on the nature and true concentrations of "free," involatile transport or leachate organotin species becomes available without recourse to chemical treatment.

Comparison of Analytical Results from GC-FPD and HPLC-GFAA

Before turning to our current organotin speciation studies in the Chesapeake Bay estuary, it is useful to compare the nature of experimental data, their form, and relative reliability, which, in turn, depend on the physico-chemical differences between the two methods. Figures 4 and 5 compare calibration curves for organotins from both anthropogenic and environmental sources. System detection limits, δ (95 percent confidence level)[51], for biocidal triorganotins representative of commercial pesticides in widespread use [13] are shown in figure 4. Mixtures of R_3Sn^+ compounds ($\text{R} = n\text{-butyl, phenyl, cyclo-hexyl}$) were separated by ion exchange HPLC-GFAA [49]. The small spread in calibration slopes in figure 4 signifies similar efficiencies for their separation and column recovery, as well as GFAA sensitivities. The δ values (ng) shown in the figure indicate that with 100 μL sample injections, nominal solution working ranges of 50 to 2,600 $\mu\text{g L}^{-1}$ may readily be speciated and quantitated. As seen in figure 5, considerably more sensitivity is possible with P/T-GC-FPD speciation of related organotin species known [5,10,11] to occur in environmental media. Much greater divergence in the P/T-GC-FPD system calibration slopes (ratios > 25) is obtained, probably a result of different hydridization rates during the fixed P/T purge time (10 min), different partition coefficients affecting sparging rates of each species [12], or different retentivities on the Tenax-GC sorbent [46]. On the basis of the δ values obtained for the GC method (fig. 5), with 10 mL sample volumes usually employed in our laboratory, nominal working ranges of 10 to 40 ng L^{-1} organotin are feasible.

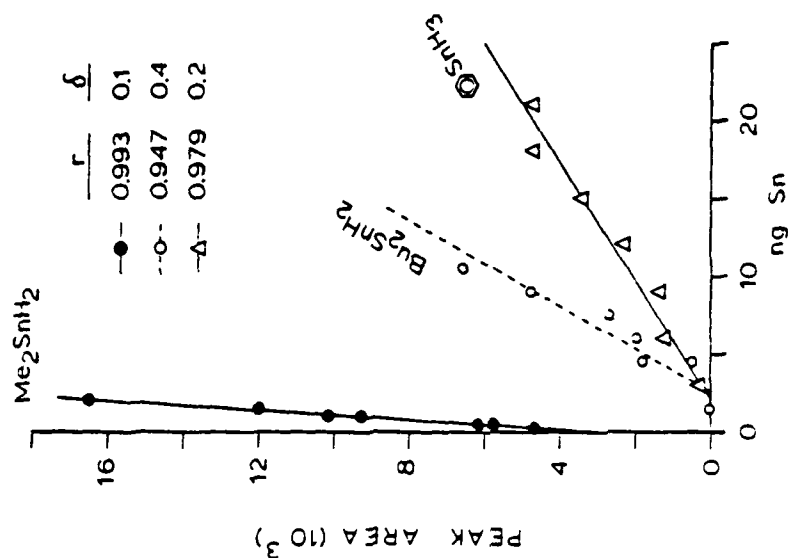


Fig. 5. Calibration curves for three aqueous organotins indicated by the hydride generation P/F-GC-FPD using the hydride generation mode [12] are shown with respective r and δ estimates.

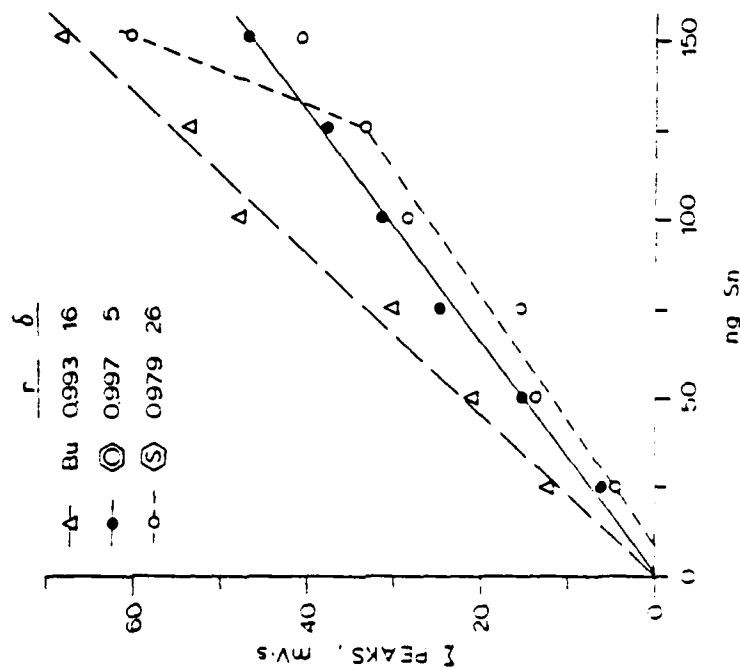


Fig. 4. Calibration curves for R_3Sn^+ (R = butyl, phenyl, n -hexyl) separated by HPLC-GFAA with strong cation exchange (SCX) columns using MeOH/H₂O/NH₄OAc eluents [40] are shown with respective correlation coefficients (r) and system detection limits (δ) (95% confidence level [51]).

Both systems are capable of at least a ten-fold increase in sensitivity with only minor changes in procedure and equipment. For HPLC-GFAA, this can be achieved by both increasing injected sample size and optimizing flow rates with a GFAA thermal program designed to give maximum atomization efficiency for a specific organotin analyte [50,51]. For P/T-GC-GFD, improvements are realized by adjusting purge flow rate and time while altering NaBH_4 additions to optimize evolution of a given organotin analyte [12]. Also, both increasing sample volumes [10,11] and operating the Tenax-GC trap at sub-ambient temperatures [2] will yield lower working ranges.

Ultimately, such concerns for widely different system responses to various organotin analytes are very crucial to reliable environmental speciation and quantitation by coupled chromatographic-element-selective detection methods because: (a) each known species analyzed must have its (same or different) calibration curve established, and (b) unknown species must either be unequivocally identified and calibrated or their calibration curves inferred from similar molecular types or guessed. Under ideal conditions, of course, a perfect chromatograph-detector system will always yield identical calibration curves for any separated molecule containing the diagnostic element of interest [17,51]. It should be recognized that in some circumstances where robust chemical derivatization is called for, as with formation of volatile tin hydrides from sea water for FPD measurements (equation (5)), possibilities exist for unexpected chemical transformations of unknown substrates into forms taken as characteristic of known analytes. In concluding this section, we will present an example for the tin(II)-tin(IV) redox system which we now feel casts uncertainty on the reported speciation and quantitation of tin as Sn(IV) in marine waters [10,11].

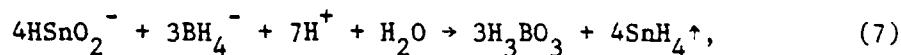
The Question of Sn(IV) and Sn(II) Speciation in Aquatic Media

Most workers regard "free" and particulate-bound tin in the sea to be in its +4 oxidation state. Based upon available free-energy data [52] and usual oceanic values of $\text{pH} = 8.1$ and $\text{pE} = 12$ ($E^\circ = 0.7394 \text{ V}$) [53], the thermodynamic distributions of supposed principal tin species are given by the following equilibria:

$$\log_{10} \frac{[\text{SnO}_3^{2-}]}{[\text{HSnO}_2^-]} = 37, \text{ and } \log_{10} \frac{[\text{SnO}_3^{2-}]}{[\text{Sn}^{4+}]} = 45. \quad (6)$$

In anaerobic environments (reducing conditions), Sn(II) and other tin(IV) species, including chloro-hydroxy derivatives [5,32], may become important. Additionally, we recognize that the steady-state condition and concentrations of Sn(II) and Sn(IV) in aquatic systems may be kinetically, not thermodynamically, controlled by biological activity. This was found to be the case for observed non-thermodynamic equilibrium concentrations favoring arsenite over arsenate produced by bacterial reduction [54,55] coupled with relatively long lifetimes of As(III) in aerobic waters [56]. We are, therefore, left with the possibility that in some marine waters, especially highly polluted estuarine sites, that Sn(II) species may occur. The question arises: does our current use of hydride generation insure that the SnH_4 generated from aquatic samples is a faithful representation of the presence only of Sn(IV) species or can Sn(II) interfere with and obscure the quantitation?

Concern for substantial interference by Sn(II) in the reduction of $\text{Sn}^{4+}_{\text{aq}}$ via equation (5) is justified. Schaeffer and Emilus showed [57] that yields of SnH_4 as high as 84 percent (based on Sn) were obtained from BH_4^- treatment of stannous chloride in aqueous 0.6 N HCl. Yields were dependent on pH, and smaller yields of distannane were noted. Jolly and Drake subsequently refined this procedure to provide a preferred general method for aqueous generation of Ge, As, Sb, and Sn hydrides [58]. For tin(II) the reaction



involves oxidation of the metal to Sn(IV) with concurrent redox of H^- and H^+ (possibly as $\text{H}\cdot$). The basic problem for our present methods employing borohydride treatments of water samples for Sn(IV) "speciation" is clear. In a recent series of experiments we have attempted to determine the extent of the problem. Our results are summarized in table 4.

Calibration curves were generated by the P/T-GC-PFD method for borohydride reductions of Sn(IV), Sn(II), and $\text{Me}_2\text{Sn}^{2+}$ species to SnH_4 , SnH_4 , or Me_3SnH_2 , respectively, in three types of media, as tabulated. All three analytes showed substantial increases in their calibration slopes in going from distilled water to 0.2 M NaCl

Table 4. Hydration of Sn(IV), Sn(II), and $\text{Me}_2\text{Sn(IV)}$ in Aqueous Media

Species ^a	Medium	Detection Limit, ng L ⁻¹ ^b	Regression Slope \pm S.E.	Number of Runs, N	R	Relative Sensitivity ^c
Sn(IV)	H_2O ^d	19	210.4 ± 16.4	7	0.985	1.00
Sn(II)	"	36	32.6 ± 4.9	7	0.949	0.16
$\text{Me}_2\text{Sn}^{2+}$	"	18	5322.0 ± 382.0	7	0.987	25.3
Sn(IV)	0.2 M NaCl ^d	29	318.5 ± 40.2	10	0.942	1.51
Sn(II)	"	23	117.9 ± 9.6	11	0.972	0.56
$\text{Me}_2\text{Sn}^{2+}$	"	21	$10,997.0 \pm 1016.0$	10	0.968	52.3
Sn(IV)	Bay water ^e	30	21.6 ± 2.8	10	0.937	0.10
Sn(II)	"	10	51.4 ± 2.2	9	0.993	0.24
$\text{Me}_2\text{Sn}^{2+}$	"	37	5086.0 ± 589.0	4	0.987	24.2

^aAdded as solutions of $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$, SnCl_2 , or Me_2SnCl_2 , respectively, to 10 mL P/T samples^bEstimated at 95 percent confidence level by the method in Ref. 51; ^cRatio of regression slopes;^dIn distilled, deionized water (18 MB \cdot cm); ^eIn Jones Falls, Baltimore Harbor water, pH \sim 8, $\text{SO}_4^{2-}/\text{NO}_3^- \sim 11$, pre-purged with zero N_2 (1 hr at 50 mL min⁻¹) before spiking with tin species.

solution, the latter approximating the salinity and ionic strength common to estuarine waters. Presumably, these effects could arise from formation of chlorohydroxy tin species favoring more rapid hydridization (cf. equations (5) and (7)) [5,32], as well as the more propitious partition coefficients for dynamic gas stripping of the volatile tin hydrides from saline solutions [12,46]. In the typical laboratory distilled water calibration solutions only 16 percent of Sn(II) was recovered as SnH_4 , compared with Sn(IV),

though this sensitivity ratio can probably be altered somewhat with pH changes [10,57]. However, in spiking anaerobic pre-purged Chesapeake Bay water with these three tin species, a striking reversal occurred in overall relative sensitivities, i.e., calibration slopes. We found that not only was Me_2SnH_2 generation

repressed by 50 percent, but that, very significantly, SnH_4 formation from Sn(IV) was reduced by 15-fold, as compared with the NaCl medium. Production of SnH_4 from Sn(II) was decreased by 50 percent

in going from NaCl to Bay waters. Clearly, dissolved or particulate substances in the Bay water, not involving Cl^- primarily, were responsible for severe interferences in the P/T-GC-FPD analyses of the three tin species, the most inhibitory effect being seen with the strongest Lewis acid, Sn(IV). Less diminution (about one-half)

occurred with the weaker acids, $\text{Me}_2\text{Sn}^{2+}$ and Sn(II), which either form less stable chlorocomplexes (vide supra) or are less likely to form strong bonds to ligands and substrates present in estuarine waters. The overall effect by estuarine water on the hydridization process is thus one of reducing yields of the three tin species tested. We expect that not only the dissolved and particulate organics and Cl^- influence formation of Sn-H bonds, but that other aquated metal ions play an important role, too. Several workers have reported that, for example, As(III), As(V), Cu(II), Co(II), Ni(II), Hg(II), Pb(II), and Ag(I) interfere by unknown means at low concentrations [10,59]. Several of these metals are commonly found in the polluted Baltimore Harbor waters (see table 1), and their presence may explain, in part, our results. In solutions of 0.01M

HNO_3 , we found that yields for $\text{Me}_2\text{Sn}^{2+}$ reduction with NaBH_4 were consistent with the values obtained in distilled water and pre-purged Bay water. However, stannane yields from Sn(II) and Sn(IV) in 0.01M HNO_3 diminished considerably as a result of either insufficient trapping from rapid evolution of stannane caused by HNO_3 or

of a repressed reaction caused by HNO_3 . In addressing the former consideration, cryogenic trapping would improve recovery of SnH_4 formed by hydride reduction.

In summary, the hydride generation method cannot adequately differentiate between aquated Sn(IV) and Sn(II) which may coexist in certain, especially anaerobic, environments found in marine waters. Previous reports [10,11] of inorganic tin, speciated as "tin(IV)," should probably be regarded as "total reducible inorganic tin" until more discriminatory techniques become available.

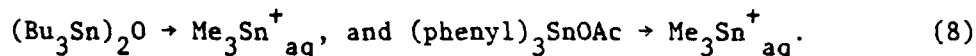
OCCURRENCE AND FATE OF METHYLTINS IN ESTUARINE WATERS

Biomethylation of Tin in Sediments

Recently, three groups have independently reported on the methylation of both inorganic tin and organotin substrates by the mixed populations of microbial flora present in sediments collected from a Canadian fresh water lake [7], and from estuarine sites in San Francisco Bay [8] and Chesapeake Bay [2,9,60]. Biogenesis of Me_4Sn was seen only to occur with additions of Me_3Sn^+ to incubated sediments [7,8], but the redistribution reactions of intermediate methyltins to form Me_4Sn by non-biological pathways must be noted as competitive events in such experiments [4,8]. The concentrations of tin compounds added to incubated sediments were consistent with values found in polluted sediments (table 1). The influence of other bioactive pollutant metals also commonly found in such sediments was not investigated. We already noted that a pure culture of a metal-tolerant *Pseudomonas* species isolated from the Chesapeake Bay [35] methylates Sn(IV) in the presence of mercury, and subsequent abiotic demethylation of $\text{Me}_n\text{Sn}^{(4-n)+}$ ($n = 3,4$) by Hg^{2+} occurs to give MeHg^+ [6]. This reaction (equation (3)) and other possible demethylation routes (cf. table 3) may importantly influence methyltin distribution patterns emanating from sediments. It, therefore, becomes very important in future incubated sediment experiments to establish the relationship between biogenic $\text{Me}_n\text{Sn}^{(4-n)+}$ distributions and the presence of Hg and other bioactive heavy elements.

Estuarine Degradation, Methylation, and Uptake of Man-made Organotins

Sediment incubation studies implied that commercial organotin biocides, particularly those used prevalently as marine antifoulants, could undergo transformations which replace existing R-Sn bonds with methyl groups [2,7]



No evidence for intermediate products, $\text{R}_n\text{SnMe}_{3-n}^+$, though plausible, was given. In one sense, this is a desirable environmental biodegradation of spent man-made biocides according to equation (2) if such processes occur in estuaries. On the other hand, the extent (rate) of conversion of R_3Sn^+ to Me_3Sn^+ is unknown. Hence methyltins formed by release of R_3Sn species from widely dispersed vessel antifouling paints cannot be discounted as an active pathway for uptake of physiologically active tin in local food chains. Preliminary evidence points to a bioaccumulation trend for total tin in bottom feeder mussels (*Mytilus edulis*) from vessel-related activities in Southern California harbors [61]. Thus, we see that bioconcentration of either man-made forms of organotins or biologically transformed organotins may occur to some degree by transfer from one trophic level to the next higher, as has been demonstrated for Hg(II) in a three compartment Chesapeake Bay food web comprising bacteria \rightarrow ciliated protozoa \rightarrow copepods [62]. Such studies involve determination of the species and the flux of organotins resulting from anthropogenic releases of organotin biocides into estuarine waters, as well as identification and assessment of the microbial flora responsible for uptake of bioactive tins into the primary trophic level of estuarine food webs.

We recently examined the form and extent of organotins leached into water from typical commercial marine antifouling paints [64] and a prototype organotin polymer formulation under development by the United States Navy as a long-term controlled release antifoulant. Such materials are regarded as sources of man-made organotin discharge into navigable waters, especially from ships at dock in harbors. In comparison, we also examined a typical blasting grit from a Chesapeake Bay shipyard where it was used to remove old or "spent" antifouling paint. Here, our aim was to speciate the chief

leachable forms of weathered or degraded organotins discharged into sluice waters in the shipyard operations or directly into harbor sediments from ships' hulls. Figure 6 depicts direct speciation of such leachable organotins by the HPLC-GFAA method. In figure 6a we see that the organotin polymer (OMP) controlled-release formulation slowly releases both biocidal triorganotin moieties as free R_3Sn^+ species in water. This chromatogram was taken from leachates in solution during the late, zeroth-order release rate exhibited by this OMP. At this point, the major release toxicant is Pr_3Sn , implying that mixed formulations of controlled-release agents may discharge their ingredients at differing rates and thereby yield variable toxic effects on target microfouling organisms and the marine environment. Below, we will suggest that steady release of these triorganotin cations may find rapid uptake on both viable and dead cells in estuarine waters.

Figure 6b offers direct evidence for degradation of Bu_3Sn -containing antifouling paints following field service on ships hulls. We cannot ascertain yet if the degradation of original Bu_3Sn^+ to Bu_2Sn^{2+} occurred by purely chemical weathering forces (light, oxidants, or marine chemicals), or by biological agents such as bacteria. The obvious important consequence is that such widespread use of these paints can discharge leachable forms of organotins into estuarine harbor locales. The tri- and dibutyltin leachates speciated here were present at 3 and 6 mg L^{-1} , respectively. No evidence for other organotin ions, such as methylbutyltin species was found [49].

Tin-resistant bacteria were recently estimated to represent 17 percent of the total bacterial population at nine diverse sites in the Chesapeake Bay [2,9,60]. We have similarly isolated 11 Bu_3Sn -resistant pure bacterial strains, including *Pseudomonas* 244 [6,35], from four stations in Baltimore Harbor and the Bay [63]. All were gram negative rods which grew on Nelson's agar [35] containing 50 mg L^{-1} tin as Bu_3SnCl or 100 mg L^{-1} tin as $Sn(IV)$. Tin uptake was measured for nine of the isolates as a function of time: at time zero Bu_3Sn^+ was added (10 mg L^{-1} final concentration) to flasks

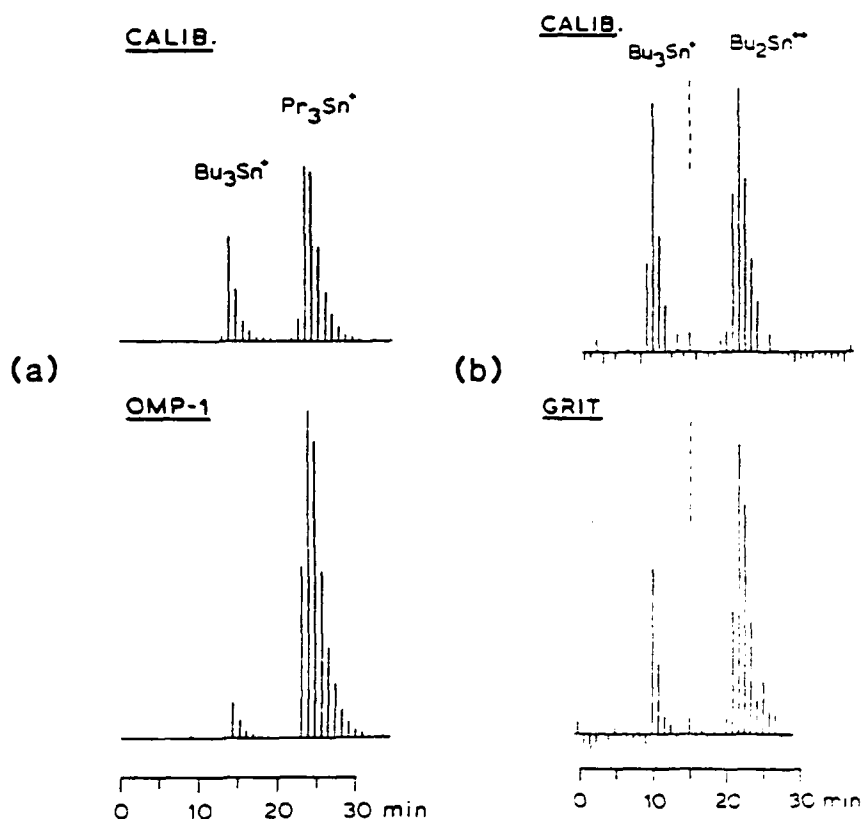


Fig. 6. Typical chromatograms obtained by tin-specific HPLC-GFAA employing a strong cation exchange column₁ with isocratic $MeOH-H_2O-NH_4OAc$ mobile phase at 1 mL min^{-1} show separations of di- and triorganotins following direct injections (100 to 200 μL) of aqueous leachates obtained from: (a) a candidate Navy antifouling co-polymer of tri-n-butyltin and tri-n-propyltin methacrylates (1:1) [64]; and (b) water shaken with shipyard grits used for removal of weathered antifouling paints [49]. Areas under chromatographic "peaks" were accurately estimated by summing all individual GFAA peak heights comprising each HPLC "peak" shown [50]. The vertical-dashed "break" in (b) signifies a flow rate change to 1.5 mL min^{-1} .

containing starved cells in buffer ($pH = 7.4$) solutions with or without glucose (10 mM). Aliquots of cell suspensions were removed after 1.5 hr, filtered, rinsed, and digested in hot HNO_3 for total

tin analysis by GFAA. For 9 isolates, the total tin uptake as a dry weight concentration factor (mg per g cells/mg per mL medium) was 621 ± 179 (no glucose) and 602 ± 274 (glucose added). This suggests that Bu_3Sn^+ accumulation was not an energy-requiring process since glucose did not stimulate starved cells to greater tin uptake. For several isolates, aliquots of cells exposed to Bu_3Sn^+ solutions were collected at more frequent intervals. Generally, we found the rate of Bu_3Sn^+ uptake to be rapid, apparently reaching steady state after 2 hr. Cell-bound Bu_3Sn^+ was substantially removed by 1 mM EDTA or methanol washes, and this procedure afforded a method for direct solution speciation by HPLC-GFAA of both the organotins taken up by cells and the residual organotins remaining in growth media. Typical results from the speciation work are illustrated in figure 7, where it was seen that virtually no biotransformation of original Bu_3Sn^+ into a logical degraded product, $\text{Bu}_2\text{Sn}^{2+}$ occurred. We also noted that killed whole cells accumulated almost twice as much Bu_3Sn^+ as live cells, suggesting that exclusion or volatilization of tin might be a mechanism of resistance and that cellular detritus in estuarine waters may be an important "sink" for organotins [63].

TETRAMETHYLTIN AND METHYLTIN HYDRIDES IN CHESAPEAKE BAY

During 1980 we surveyed unfiltered surface and bottom waters at sites in Baltimore Harbor and the Bay channel, selected for their respective decrease in anthropogenic tin influx (table 1) [2,20,21]. Employing the P/T-GC-FPD system in both modes, e.g., with or without NaBH_4 additions to replicate samples, we established [12] for the first time the presence of tetramethyltin in natural waters. We additionally demonstrated the unexpected presence of methyltin hydrides, $\text{Me}_n\text{SnH}_{4-n}$ ($n = 2,3$), also heretofore unknown in the environment. Main features and interpretation of the experimental results are illustrated by the chromatograms in figure 8.

Use of two modes with P/T analyses provides an effective tool for speciating both solvated or involatile $\text{Me}_n\text{Sn}^{(4-n)+}_{\text{aq}}$ and volatile coordinatively saturated $\text{Me}_n\text{SnH}_{4-n}$ according to equation (1).

CALIBRATION

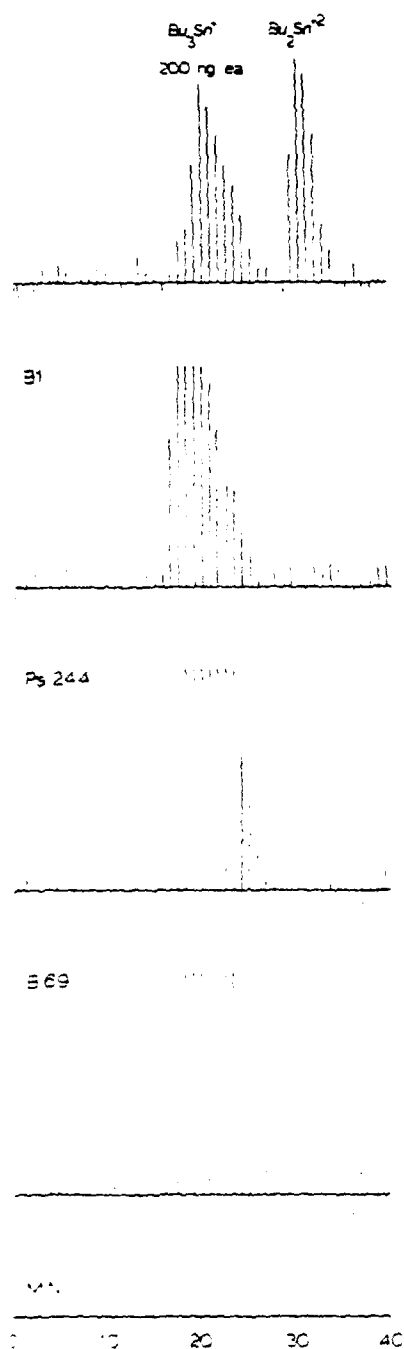


Fig. 7. HPLC-GFAA chromatograms showing speciation of cell-bound organotin on three examples of Chesapeake Bay tin-resistant bacteria are compared with a calibration run for 200 ng each of Bu_3Sn and Bu_2Sn species. The isolates were grown in Nelson's broth containing 10 mg L^{-1} Bu_3SnCl , harvested (10 mL) by ultra-centrifugation, and washed 3 times in distilled water. A methanol extract (100 μL) was injected directly into the HPLC-GFAA system.

No significant (95% confidence level) formation of dibutyltin degradation products resulting from cellular extracts (shown) or residual growth solution free of cells was observed. The highest GFAA peaks in the chromatograms represent off-scale values collected with the digitizer. Based on overall system sensitivity, biotransformation to Bu_2Sn of 1% Bu_3Sn in growth solutions or 3% of cell-bound Bu_3Sn would have been detected [63].

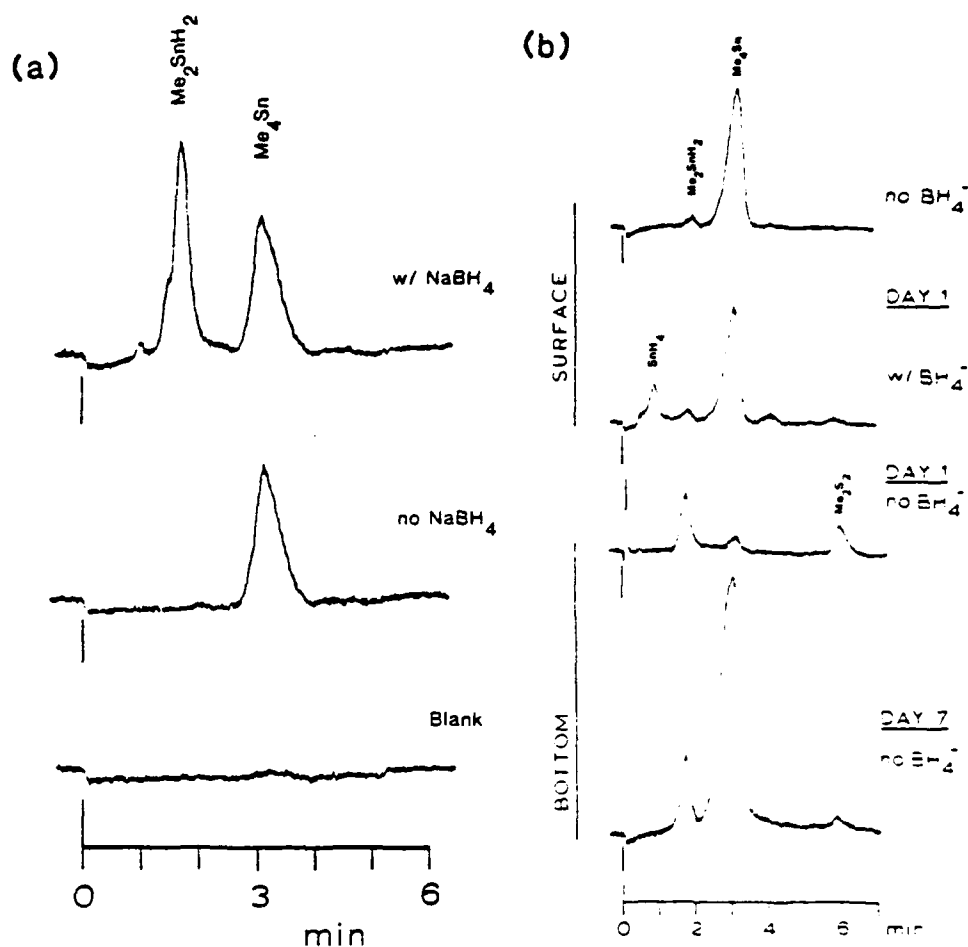


Fig. 8. Application of P/T-GC-FPD system for speciation of organotins: (a) replicate calibration solutions containing Me₂Sn²⁺ plus Me₄Sn run by Mode II (addition of NaBH₄) (top) and by Mode I (no addition of NaBH₄) (middle) are compared with blank run by Mode II; (b) representative samples from surface and bottom waters at Jones Falls sewage outfall (Baltimore Harbor), fresh (day 1) samples were stored at 2 to 4°C and run (Mode I) within 24 hr after collection, aged (day 7) sample was maintained at 2 to 4 °C then run by Mode I.

Figure 8a depicts an example of such clearcut separation, and the future useful fact that Me₄Sn is recovered virtually quantitatively after BH₄⁻ treatment. With Chesapeake Bay waters, several impor-

tant observations were therefore possible. In both surface and bottom unfiltered waters, significant differences in the involatile/volatile methyltin distributions were noted, though highly variable. In figure 8b, for example, one surface water sample from a heavily polluted urban runoff in Baltimore Harbor, comparison of replicate samples by the two P/T modes showed that both Me_4Sn and Me_2SnH_2 were present as volatile, whereas only "reducible inorganic Sn" and some $\text{BuSn}^{3+}_{\text{aq}}$ were present as involatiles. Also in figure 8b, comparison of surface and bottom waters at this station shows characteristic increases in concentrations of Me_2SnH_2 and Me_2S_2 [12], and decreased Me_4Sn in bottom water.

We have noted that reproducible results were obtained only with water samples maintained at 2 to 4 °C and analyzed by P/T methods within 24 hr following collection. As figure 8b illustrates, samples kept refrigerated for seven days undergo substantial alteration with significant increases of Me_4Sn induced by either chemical or biological methylation, or both. These results presage lines of investigation to establish optimum sampling protocols for ultratrace aquatic organotin (organometal) speciation, as well as determining the mechanisms by which continued methylation of tin occurs in vitro.

In Chesapeake Bay, we generally found increasing methyltin concentrations with increased anthropogenic tin influx (table 1), in accord with Hallas' contention [2,60]. Concentration values obtained for individual organotin species often varied more than ten-fold within the water column at a given site and from visit to visit. We found much higher (10 X) organotin concentrations in Baltimore Harbor than in the Bay channel. The highest concentrations observed over nine months (spring to fall) occurred at Harbor locations with greatest organic loadings (Jones Falls), viz.

Me_2SnH_2 ($0.20 \mu\text{g L}^{-1}$), Me_3SnH (0.40), and Me_4Sn (0.48); a maximum value of $0.93 \mu\text{g}^{-1}$ for Me_4Sn was obtained at Colgate Creek which is primarily an urban-industrial run-off site. We conducted a sustained three-week study on Baltimore Harbor in order to compare methyltin levels with those available for other American estuaries. The data are summarized in table 5.

Table 5. Methyltins in Estuarine Waters^a

Site	SnH ₄	Me-SnH ₃	Me ₂ -SnH ₂	Me ₃ -SnH	Me ₄ -Sn	Other	Ref.
Baltimore Harbor ^b							
Surface	nd ^c nd-4 ^d	nd ^c nd ^d	<0.005-0.02 ^c <0.005-0.02 ^d	nd ^c <0.005-0.01 ^d	<0.01-0.30 ^c <0.01-0.30 ^d	0.05-0.10 ^{c,e} 0.05-0.30 ^{d,e}	12
Bottom	nd ^c 0.80 ^d	nd ^c nd ^d	<0.005-0.02 <0.005-0.10 ^d	nd ^c nd-0.02 ^d	0.05-0.30 ^c nd-0.30 ^d	nd-0.10 ^{c,e} nd-0.10 ^{d,e}	
San Diego Bay							
Surface ^{d,f}	0.009-0.038	0.002-0.008	0.015-0.045	--	--	0 ^{e,g}	11
Florida Estuaries							
Surface ^{d,h}	0.009	0.002	0.002	<0.001	--	--	10

^a Concentrations in $\mu\text{g l}^{-1}$ for analytes shown; ^b Range for three collections during three weeks at two sites; ^c Mode 1, during three weeks at two sites; ^d Mode 1, no addition of NaBH₄; ^e Mode 11, addition of NaBH₄; ^f BuSnH₃; ^g Range for five sites collected over one day; ^h Bu₂SnH₂; ⁱ Average of eleven sites collected over various periods of time.

We did not detect MeSnH_3 , either as an involatile or volatile species, in Baltimore Harbor. Presumably, from its reported presence in San Diego [11] and Florida [10] estuaries at ranges below $0.008 \mu\text{g L}^{-1}$, it may have been present in the Bay waters below our P/T system detection limit ($\sim 0.005 \mu\text{g L}^{-1}$). Though butyltins were not detected in San Diego Bay, the same workers [11] detected very high concentrations of $\text{BuSn}^{3+}_{\text{aq}}$ ($1.22 \mu\text{g L}^{-1}$) and $\text{Bu}_2\text{Sn}^{2+}_{\text{aq}}$ ($1.60 \mu\text{g L}^{-1}$) in surface waters of Lake Michigan which they ascribe to atmospheric inputs or possible contamination from PVC water samplers (cf. table 2). We found BuSn species regularly in our three-week study, both as volatile BuSnH_3 and solvated $\text{BuSn}^{3+}_{\text{aq}}$. We saw a trend toward higher concentrations in surface water (table 5), though not as pronounced as in Lake Michigan. Our P/T methods employed in the present studies, did not reliably speciate $\text{Bu}_2\text{Sn}^{2+}$ or higher homologs, but it seems more likely to us that such organotins will be anthropogenic and probably introduced from antifouling paints on vessels. More detailed studies on the distribution patterns of the entire sequence $\text{Bu}_n\text{Sn}^{(4-n)+}$ (equation (2)) based on water movements and shipping patterns are now required to resolve this point.

Finally, we can estimate the ratio of organotins to total tin in the suspended particulates found in Baltimore Harbor surface water. Nichols observed [20] that unfiltered surface water from this locale contained $0.19 \mu\text{g L}^{-1}$ (with $8.7 \mu\text{g g}^{-1}$ Sn in filtered particulates) (table 1). Our estimates of total organotins, including methyl- and butyltins for Baltimore Harbor are represented by the low values of the ranges tabulated in table 5. Thus we derive $<0.07 \mu\text{g L}^{-1}$ as an upper sum which, in turn, implies that $[(<0.07) \times 100]/(0.19) = <37$ percent of Baltimore Harbor surface water suspensions occur as organotins. This remarkable extent of bioactive tin materials seems large, and may reflect the deficiency of adequate field data at this time. In any event, both the means and needs to further explore this question are at hand [13]. In neither Nichols' [20] nor our present studies has the surface micro-layer been examined. This oleaginous film also supports amplified microbiological activity and amplifies concentrations of heavy metals [5,28]. "Free" or volatile organotin species would be expected to partition into this lipophilic layer, there to be accumulated, transformed, and possibly released into the atmosphere.

CONCERNING AQUATIC METHYLATION AND HYDRIDIC METAL (TIN) CYCLES

From the foregoing discussion of our recent results and those from other laboratories, we see that tin joins a growing family of bioactive elements for which evidence of biological and chemical methylation supporting global transport cycles widens [5,64]. In large part, substantial advances in ultratrace molecular speciation methodology and accessible commercial equipment [17,45] have spurred many creative efforts to comprehend both the biological and chemical factors that govern the likelihood, forms, and lifetimes of such organotins in the aquatic environment. We can now reasonably ask what will be the shape of future advances, and what are the road-blocks we face?

Though exciting new biogenic organometals, such as the methyl-tin hydrides in Chesapeake Bay, will continue to be unexpectedly discovered, more rigorous and predictive considerations must guide future progress. Recognition [5,14,15,26,64] that many aquatic organometallic reactions occur by second-order, or at least pseudo first-order, kinetic pathways limits any selection of useful model reactions. Moreover, we cannot presume that the wealth of chemical literature for organometallic reactions, typically obtained at high concentrations in non-aqueous solvents, can be successfully translated to environmental action levels extant at $\ll 10^{-10}$ M in aqueous saline media (cf. table 5). With current speciation techniques of the sort described in this paper, the limits of detection of reaction products of model reactions is perhaps 4×10^{-11} M, i.e., 5 ng L^{-1} as $\text{MeSn}^{3+}_{\text{aq}}$. For example, in aqueous oxidative additions of MeI to $\text{Sn}^{2+}_{\text{aq}}$ or $\text{Pb}^{2+}_{\text{aq}}$, which are bimolecular S_N2 reactions (table 3) we estimate $k_2 < 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$ [5,7,26,39]. Thus, for this tin methylation, starting with both reactants at 10^{-6} M, kinetic measurements could begin after 11 hr during which time the reaction will have proceeded only 0.004 percent; starting with 10^{-8} M reactants, product MeSn^{3+} could be detected after 0.4 percent reaction, but this would now require 12.7 years! Clearly, the need for careful selection of reaction conditions is paramount given the constraints of available detection limits for tin species or those of any other metal or metalloid.

We must also consider the thermodynamic bases upon which we assess relative populations or stability domains for aquatic ultra-trace metal-containing molecules. Figure 9 presents a highly simplified two-electron redox cycle for an element E in the chemical or biological synthesis (or degradation) of methyl- or hydrido-derivatives in an aquatic system. Here, either methyl carbanion (Me^-) or carbonium ion (Me^+) and hydride (H^-) or proton (H^+) can act as two-electron carriers in both enzymatic [65] or abiotic [15,26] reactions. The relative stability of a given element toward redox is indicated by the usual thermodynamic expression for $E_{\text{ox}} = E_{\text{red}}$ shown as a vertical potential gradient. Approximate equilibrium concentrations for the respective species, viz. As(V) - As(III), Sn(IV) - Sn(II), or Hg(II) - Hg(0), can thereby be estimated, given appropriate environmental parameters of pH, E_h , pCl, etc. However, such calculations fall far short of approximating the true nature and concentrations of redox species in natural environments [52,55]. The relative concentrations of organometallic and inorganic species are greatly determined by non-equilibrium, catalytically induced kinetic processes which involve both chemical and biological factors [54].

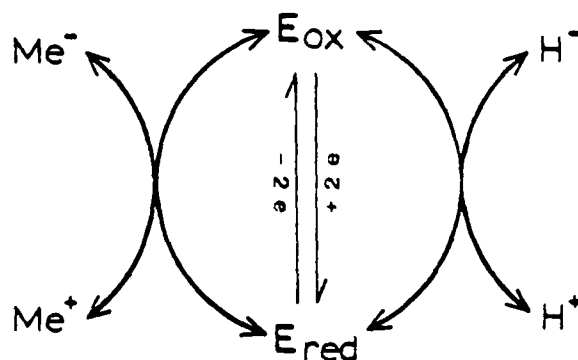


Fig. 9. Basic features of aquatic synthesis of methyl- and hydrido-derivatives of elements (E), and their possible stabilities are summarized in a two-electron redox cycle.

For a methylation (electrons added, \uparrow) or a demethylation (electrons removed, \downarrow), as well as an analogous hydridization, the crucial knowledge about the stability or lifetime of such species formed is prerequisite to assessing the extent of redox. Beyond this, knowledge about lifetimes of such methylated/hydridized metal species provides guidance for designing relevant approaches to their speciation in active environmental compartments. For example, we expect methylation of mercury(II) to result in formation of relatively long-lived [5] $\text{MeHg}^+_{\text{aq}}$ species which we can (and do) detect in marine waters, whereas any possible HgH^+ , HgH_2^0 , or MeHgH^0 species are not expected [66] to survive sufficiently long for analysis (speciation), but eject H_2 or MeH to form Hg^0 [36].

For many other metals and metalloids, such mobile metabolites can exist in natural waters for periods long enough for environmental speciation. Our discovery of methyltin hydrides, $\text{Me}_n\text{SnH}_{4-n}$ ($n = 2, 3$), in polluted Chesapeake Bay waters [12] is one good example, as was the detection of bacterial formation of methylarsines, $\text{Me}_n\text{AsH}_{3-n}$, in soils [34]. At the concentrations involved, and in the presence of a multitude of potential reactants, we conclude at this point that such hydrides may possess aquatic lifetimes far longer than suggested by the meager literature available. To a great extent, future progress in this new and important area of trace metals in the sea will depend upon careful measurements of such species' lifetimes in saline solutions and characterization of the pathways for their disappearance.

ACKNOWLEDGEMENTS

Contributions from the National Bureau of Standards are not subject to copyright. Major portions of this work were supported by the Office of Naval Research, to whom we are grateful for sustained interest and encouragement. We thank the Chesapeake Bay Institute and Johns Hopkins University for providing ship time on the R/V Ridgely Warfield. Our colleagues, R. R. Colwell, J. J. Cooney, J. M. Bellama, L. E. Hallas, Y. K. Chau, K. L. Jewett, M. Nichols, and W. M. Coleman were generous with ideas and comments, and graciously shared unpublished data with us; we thank them for their invaluable help. Identification of certain commercial equipment or materials in this paper does not imply any endorsement of quality or recommendation by the National Bureau of Standards. Rather, such specifications are made only to adequately detail experimental procedures.

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	AD A100587	9
4. TITLE (and Subtitle)	5. TYPE OF REPORT & PERIOD COVERED	
Ultratrace Speciation and Biogenesis of Methyltin Transport Species in Estuarine Waters.	Interim Technical Report.	
7. AUTHOR(s)	6. PERFORMING ORG. REPORT NUMBER	
F. E. Brinckman, J. A. Jackson, W. R. Blair, G. J. Olson, W. P. Iverson	S-5610406-TK-51	
	8. CONTRACT OR GRANT NUMBER(s)	
	N00014-81-F-0013 NR 083-471	
9. PERFORMING ORGANIZATION NAME AND ADDRESS		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
Chemical and Biodegradation Processes Group Chemical Stability and Corrosion Division National Bureau of Standards, Washington, D.C. 20234		
11. CONTROLLING OFFICE NAME AND ADDRESS		12. REPORT DATE
Department of the Navy Office of Naval Research Arlington, VA 22217		
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		13. NUMBER OF PAGES
		12 48
		15. SECURITY CLASS. (of this report)
		Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report)		
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Submitted as invited chapter in: NATO Advanced Research Institute on "Trace Metals in Sea Water", Plenum Publishing Corp., New York Paper was presented to the NATO Advanced Research Institute on "Trace Metals in Sea Water" at Erice, Sicily, Italy, March 30 - April 3, 1981.		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)		
Atomic absorption detector; biomethylation; Chesapeake Bay; estuarine organotin transport; flame photometric detector; gas chromatography; kinetics; liquid chromatography; methyltin hydrides; purge and trap sampling; redox; tetramethyltin.		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number)		
Environmental tin, widely dispersed at low concentration in waters, sediments and biota, is bioactive element susceptible to methylation and even hydridization by marine bacteria. Nonetheless, the redox cycle of tin in natural waters is poorly understood and recent advances in tin-specific molecular characterization fail to speciate Sn(II) and Sn(IV) reliably. Rapid developments in speciation methodology now permit studies of organotin distributions in aquatic systems, raising the question of the "natural" biogeochemical flux of methylstannanes in relation to increased anthropogenic organotin influx from industry and shipping. New methods		

Number 20 (continued) - Abstract

for direct speciation of aquated or involatile organotins by liquid chromatography are complimented by advances in purge-and-trap sampling of volatile organotins speciated by gas chromatography. Work from our laboratory indicates that models for estuarine formation and transport may ultimately be developed, but that basic roadblocks to progress stem from inadequate descriptive aqueous organometallic chemistry and knowledge of critical kinetic parameters for the lifetimes of key organotin species in sea water.